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ALGAL BIOASSAYS OF INDUSTRIAL AND ENERGY PROCESS EFFLUENTS[1]

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ABSTRACT

This review presents laboratory data and their interpretation with regard to effects of pollutants on marine and freshwater unicellular algae. Stimulation and inhibition of growth are considered to be equally undesirable. It is suggested that a search for new species for use in toxicity tests be made because the widely-used green alga, Selenastrum capricornutum, may not be one of the most sensitive freshwater species. Several algal species should be used to test each toxicant because there is not a single most-sensitive species and the conditions under which species are tested affects response. The batch method is the most commonly used exposure system for defining relative effects of pollutants, but mixed-species continuous culture presents a more realistic approach to estimation of effects in natural systems. Algae are more sensitive to industrial and energy-process wastes than animals. They respond to growth stimulants and growth inhibitors, and the stimulation response occurs at concentrations much lower than those that inhibit growth.

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INTRODUCTION

Unicellular algae are ubiquitous in aquatic ecosystems, where they incorporate solar energy into biomass, produce oxygen that is dissolved in water and used by aquatic organisms, function in cycling and mineralization of chemical elements, and serve as food for herbivorous and omnivorous animals. When they die, they sink to the sediment, where their chemical constituents are transformed, solubilized, and recycled into the water. These functions are dependent upon phytoplankton population dynamics which, in turn, depend upon seasonal variability in temperature, intensity of solar radiation, nutrient concentrations in the water, and grazing by animals. Natural and anthropogenic alterations of water quality can upset the balance of these controlling factors and bring about changes in species composition of the algal community, rates of production, biomass, and water chemistry. If water quality is altered by toxicants or growth stimulants from industrial, agricultural, or municipal sources, normal algal function may be upset, causing gross changes in structure and function of the receiving aquatic ecosystem.

A large amount of evidence indicates that industrial and energy process effluents contain algal and animal toxicants and algal growth stimulators. Algal laboratory tests are excellent indicators of pollution by these complex wastes. Like animals, algae in the laboratory respond to toxicants, but they also respond in an easily measurable way to substances that affect primary productivity. Algal population growth in the laboratory is rapid and may be measured directly in a variety of ways. In addition, rates of physiological

processes within exposed populations may be estimated indirectly by measurement of evolution and uptake of oxygen.

This review presents laboratory data and their interpretation with regard to effects of complex wastes on marine and freshwater unicellular algae. The scientific literature contains relatively little data on algae and complex wastes, and specific methods need to be developed for field and laboratory studies. We have, therefore, included data from tests with organic compounds and heavy metals because both are present in complex wastes. Methods used in toxicity tests with these pollutants are discussed with regard to their application to studies on complex wastes. Inhibition and stimulation of algal population growth are considered to be equally undesirable because any deviation of algal productivity or community composition from that which is usual for an aquatic ecosystem threatens the entire system.

ALGAE AS BIOASSAY ORGANISMS

Algae comprise a large and heterogeneous group of benthic and planktonic species that occur together in often complex communities. Members of the following classes are often prominent in natural assemblages: Chlorophyceae (green algae), Bacillariophyceae (diatoms), Cyanophyceae (blue-green algae), Rhodophyceae (red algae), Chrysophyceae (golden brown algae), Haptophyceae, Craspedophyceae, Xantophyceae, Prasinophyceae, Pyrrophyceae (dinoflagellates), Cryptophyceae, and Euglenophyceae. Green algae, blue-green algae, and diatoms are commonly dominant in algal communities, but toxicity tests on species from the other classes are needed in order to have a more comprehensive idea of effects of toxicants on algae.

Although there is a large number of algal species in fresh and salt water, and many of them have been used in tests with pesticides, heavy metals, and other single toxicants, only a few have been tested for response to complex wastes. In freshwater toxicity tests, Selenastrum capricornutum Printz (now called Monoraphidium capricornutum Printz (Nygaard)) is used extensively to detect the presence of growth inhibitors and growth stimulators in complex effluents (Miller et al., 1978; Walsh et al., 1980). Other species commonly used and responsive to pollutants include Chlorella homosphaera, Ankistrodesmus falcatus, and Oscillatoria agardhii (Claesson, 1975), Nitzschia linearis (Patrick et al., 1968), and Euglena gracilis (Falchuk et al., 1975a and b.).

Palmer (1969) listed 60 genera and 80 species of freshwater algae according to tolerance to pollutants. Although members of each group varied widely in response, green and blue-green algae generally were most tolerant and diatoms least tolerant to pollutants. The five most tolerant genera were Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, and Chlorella and the five most tolerant species were Euglena viridis, Nitzschia palea, Oscillatoria limosa, Scenedesmus quadricauda, and Oscillatoria tenuis. The five most sensitive genera were Crucigenia, Cymatopleura, Dictyosphaerium, Selenastrum, and Stauroneis, and the five most sensitive species were Tetraedron muticum, (now Goniochloris mutica (A.Br.) Fott), Pyrobotrys gracilis, Euglena proxima, Gonium pectorale, and Cryptomonas ovata. No species and only one genus, Selenastrum, listed above as pollution-sensitive is used in laboratory toxicity tests.

It is possible that S. capricornutum is not the most sensitive fresh-water algal species to pollutants in laboratory toxicity tests. Shiroyama et al. (1975) stated that S. capricornutum, although not indigenous to the United States, was chosen for use in the algal assay procedure bottle test because it: is easy to culture and identify, displays minimum morphological changes during the population growth phase, is single-celled and thus can be enumerated on an electronic particle counter, and is widely distributed in eutrophic and oligotrophic waters. None of these characteristicss addresses the question of sensitivity to pollutants. Rodhe (1978, cited in Miller et al., 1978) stated that Selenastrum belongs to a group of ubiquitous algae that have a wide range of tolerance toward environmental conditions. Slobodkin and Sanders (1969, cited in Fisher et al., 1973) postulated that organisms adapted to stable environments would, in general, be more sensitive to environmental stress than organisms adapted to less stable conditions. Besides being more resistant to environmental change, species adapted to unstable conditions may adapt to culture conditions and thus their physiological responses may not be similar to those in the field. Highly adaptable species may adapt to the presence of bioactive substances from waste outfalls in laboratory tests. It is possible that only the most hardy species may be cultured in the laboratory and that species sensitive in natural systems cannot be evaluated in standard laboratory tests.

There is a need for screening studies to search for new, more sensitive freshwater species that respond to both growth inhibitors and growth stimulators. Many new algal species can be cultured and tested in the laboratory, and the more sensitive species might be isolated from geographical

areas of interest. Care must be taken, however, not to sample from contaminated waters because of the possibility that resistant forms will be collected.

Culture collections often have clones that can be tested. For example, the Culture Collection of Algae at the University of Texas at Austin lists as available: Tetraedron (5 clones), Pyrobotrys (1 clone), Crucigenia (2 clones), Dictyosphaerium (4 clones), Selenastrum (6 clones), Stauroneis (1 clone), Euglenia proxima (1 clone), Gonium pectorale (6 clones), and Cryptomonas ovata (1 clone).

A large number of marine algal species have been tested with complex effluents. For example, Dunstan (1975) used 15 species to analyze effects of secondary treated sewage, Brack et al. (1976) used four species to describe synergistic and antagonistic effects of heavy metals, and Thomas et al. (1980a) reported effects of mixtures of ten heavy metals on four species of marine algae. Rainville et al. (1975) used Coccochloris elebans because it is common in the marine phytoplankton, reacts rapidly to environmental change, responds to toxic substances, is genetically stable, and grows rapidly in suspension in laboratory cultures.

Before new species can be used in tests, their characteristics in laboratory culture must be defined to be certain that they do not depart greatly from those of natural populations. For example, original bioassay protocols for eutrophication studies (U.S. Environmental Protection Agency (EPA), 1971) recommended Microcystis aeruginosa and Anabaena flos-aquae as test species. Wyatt (1973), however, pointed out that laboratory cultures of these cyanophytes differ greatly from natural populations. In culture,

M. aeruginosa does not take the form of a gelatinous colony and neither M. aerugiosa nor A. flos-aguae forms gas vesicles as in nature. Laboratory responses to toxicants with such atypical clones cannot be considered as estimates of responses in natural systems. On the other hand, morphological changes caused by pollutants in laboratory tests may be important detection tools and are discussed later.

Algal clones have been shown to respond differentially to pollutants. Fisher et al. (1973) showed that stenothermal and stenohaline clones of Thalassiosira pseudonana, Fragilaria pinnata, and Bellerochia sp. from the Sargasso Sea were more sensitive to PCBs than clones of the same species from estuaries and over the continental shelf. Jensen et al. (1976) demonstrated that two clones of Skeletonema costatum responded with equal sensitivity to copper ions, but differed markedly in response to zinc. It may be necessary, therefore, to test clones of many species in order to find those most sensitive in most tests.

Although algal tests are usually performed to detect toxicity, lack of toxicity is just as important to pollution-control workers. Thomas et al. (1980b) used an algal toxicity test with Asterionella japonica, S. costatum, Chaetoceros affinis, Gymnodinium splendens, Gonyaulax polyhedra, and Dunaliella sp. to demonstrate that ammonium from a sewage outfall was not toxic to the algae. However, growth was stimulated, and the authors concluded that there is probably a balance between inhibitory and stimulating factors put into receiving waters by outfalls.

Most algal tests employ only one species, whereas algae occur in nature in often complex communities whose compositions change throughout the year. Although single pollutants are known to affect competition among algae, virtually nothing is known about effects of complex wastes on species composition or function of unicellular algal communities. Titman (1976) showed that the relative abundance of phosphate and silicate determined the relative abundance of Asterionella formosa and Cyclotella meneghiniana. Tilman (1977) demonstrated how the functional resource acquisition and/or utilization responses of A. formosa and C. meneghiniana in the presence of limiting amounts of phosphate and silicate regulate species numbers in mixed culture. Lewis (1977) showed that rare algal species are as likely to have an opportunistic strategy as common species and that filamentous algae are likely to be opportunistic (i.e., species that have marked power of increase under a narrow range of favorable conditions), whereas coenobial types are conservative (i.e., lack the ability to produce impressive growth surges but persist more effectively when conditions are unfavorable). It is not known how complex wastes may affect competition among algal species; whether by alteration of available resources, direct effects on the algae, or both. Research is needed on effects of pollutants on species composition of algal communities. Effects in culture must be related to field conditions such as temperature: Goldman and Ryther (1976) showed that results of competition are highly dependent on temperature.

It is probable that species in unialgal culture respond to pollutants in a manner different than they would if exposed in multispecies tests. Gamulin-Brida et al. (1967) studied subtidal algal communities and concluded that single species in culture do not respond to physical and chemical conditions of the environment with the same sensitivity as when in competition with other species in natural situations. Fielding and Russell (1976) found this to be true in vitro when the marine macrophytic algae Ectocarpus siliculosa, Erythrotricia carnae, and Ulothrix flacca were exposed to copper singly and in combination. There were two types of algal reactions: competition and annidation (i.e., one species is stimulated by the presence of the other). For example, U. flacca grew better than E. siliculosa when grown together in untreated control culture. In the presence of 50 parts per billion (ppb) copper, growth was equal, but in 100 ppb, E. siliculosa grew better than U.flacca. At 100 ppb Ulothrix enhanced the growth of E. carnea, which enhanced the growth of E. siliculosa. Fielding and Russell (1976) suggested that, in these cases, copper might have been complexed by extracellular products of algal metabolism or taken up by living or dead cells.

Fisher et al. (1974) demonstrated strong effect of 0.1 ppb PCB on natural phytoplankton assemblages in a flow-through continuous culture system. Species diversity was reduced strongly after 146 hr exposure, and the authors suggested that the toxicant might alter the natural state of interspecific competition and decrease the diversity of natural communities. Goldman and Stanley (1974) studied growth interactions of Dunaliella tertiolecta, Thalassiosira pseudonana, and Phaeodactylum tricornatum in

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wastewater-seawater mixtures and suggested that a complex interaction of environmental factors is usually responsible for dominance of a species. The source of nitrogen was important in their studies. When the source was NO_3 - N, the carbon:nitrogen ratio in the algae was high (7 to 8), when NH_4 - N, the ratio was low (4 to 6).

It is clear that the species of alga and the conditions under which it is tested are important when estimating effects of pollutants on algae and algal communities. Most toxicological research with algae has been done with single pollutants such as pesticides and heavy metals, but it is not known whether species most sensitive to single pollutants are also most sensitive to mixtures. There is a need, therefore, for screening tests with algae and complex wastes to determine which species and conditions are best for detection of pollution from industrial outfalls. The following suggestions may be used as criteria for establishment of a testing program:

1. Use ecologically important sensitive species.
2. Measure the most important end-point for each species, i.e., inhibition and/or stimulation of growth, rates of photosynthesis and respiration, etc.
3. Use mixed-species tests to determine effects on communities, i.e., changes in dominance and rates of primary production.
4. Use experimental conditions that relate to species survival and abundance and community structure under varying conditions found in natural systems, i.e., temperature, light, and salinity.

Use of indigenous species may not be protective of the environment because pollutants are transported easily to other areas. Use of sensitive species is much more important than use of indigenous species.

The above criteria imply knowledge of the population dynamics and physical and chemical requirements of sensitive algal species. Such knowledge can be used in multifactorial laboratory tests to produce algal toxicity data that relate to field conditions.

ALGAL TOXICITY TESTS

A variety of tests has been developed for measuring effects of toxicants and growth stimulators on algae. Although they vary according to composition of growth medium, species tested, size and shape of exposure vessel, intensity and cycle of lighting, etc., most are static tests with single species in enriched culture medium. Continuous culture techniques or flow-through tests have been used with monospecific and mixed species cultures, but to a smaller degree than static batch tests. Both static and flow-through tests estimate growth-rate responses by measurement of change of biomass, chlorophyll content, cell numbers, or fluorescence. Rates of primary production have also been estimated by measurement of oxygen evolution or uptake of ^{14}C.

Field Tests

Algal tests have been used in the field and laboratory to determine biological potential of natural waters and of substances from industrial, municipal, and agricultural sources dissolved in them. They have also been used to directly estimate potential effects of waste streams. Schultz and Gerhardt (1969) described design and use of dialysis culture, and Trainor (1965), Jensen et al. (1972), Prakash et al. (1973), and Jensen and Rystad (1973) described methods of dialysis culture that can be used in the field

and laboratory. Sakshaug and Jensen (1978) reviewed use of dialysis cultures for the study of marine phytoplankton and stated that such culture extended conventional bioassay methods because:

1. Dialysis cultures can be easily grown *in situ*.
2. Algal accumulation of trace compounds from large volumes of medium is possible.
3. Dense populations can be obtained in oligotrophic waters.
4. Control of one or more growth requirements is easily established.
5. Growth rates and intrinsic algal properties can be monitored as functions of environmental factors, including pollutants.

In dialysis culture, algae are retained in dialysis bags or in vessels fitted with dialysis membranes and exposed to soluble pollutants or nutrients in natural waters that can pass through the pores of the bag or membrane. Growth of the algae can then be measured spectrophotometrically, by cell count, by direct gravimetric analysis of biomass, or by chlorophyll analysis. Jensen *et al.* (1972) demonstrated effects of non-enriched seawater, depth, and nutrients on growth of eleven species of marine unicellular algae by suspending dialysis bags with algae in flowing seawater in the laboratory or from a buoy in the open sea. Dor (1975) used dialysis culture to demonstrate stimulatory effects of soluble substances in domestic sewage on the freshwater species, *Scenedesmus obliquus*. Powers *et al.* (1976) exposed the marine dinoflagellate, *Exuviella baltica*, to low concentrations of DDE in dialysis bags and incubated them in a salt marsh. Population growth in bags in the field was inhibited as compared to laboratory and field untreated control cultures. Among control cultures, populations in

dialysis bags suspended in the water of the salt marsh grew at a faster rate and attained greater biomass than did laboratory populations cultured in natural water or enriched seawater.

Although it has not been used extensively, and field data need to be compared to laboratory results, dialysis culture is potentially an excellent method for estimating effects of soluble components of industrial and energy process effluents on receiving water. Unialgal or mixed algal cultures could be placed at an outfall and at selected sites downstream to measure effects on growth, species composition of test communities, rates of photosynthesis and respiration, or chlorophyll concentration. Such field tests would be easy to do and would relate more closely to natural conditions of light, temperature, and nutrient availability than laboratory tests.

Batch Culture

In freshwater, the most commonly-used method for assaying the algal growth supporting potential of natural waters is the algal assay procedure: bottle test (EPA, 1971; Miller et al., 1978). This method, which uses flasks instead of bottles, was developed to detect nutrient status of natural waters. One parameter used to measure effect in the bottle test is the maximum standing crop in each flask, which is determined gravimetrically or indirectly from cell counts and cell volume as determined with an electronic particle counter. Miller et al. (1978) recommended a growth period of 14 days or until the increase in biomass is less than 5% per day. Greene et al. (1975) used the method to detect effects of nitrogen and phosphorus from a primary sewage treatment plant on algal growth. The standard algal assay bottle test estimates effects of substances dissolved in natural waters by

expression of population growth rate and dry weight biomass. Cain and Trainor (1973) modified the method to measure population growth by changes in optical density of algal cultures, transforming optical density data to cell number. They also added test water to their cultures each day to minimize effects of nutrient carryover after luxury uptake to determine growth rates under conditions of nutrient replenishment, as occurs in rivers. They demonstrated effects of a sewage treatment plant on the nutrient carrying capacity of a river and stated that, although final yield in cultures to which medium is not added each day is a function of available nutrients, such data may be of less value than growth rate data obtained under conditions of nutrient replenishment. Cain et al. (1979) compared results of freshwater algal tests to chemical analyses of effluent from a sewage treatment plant and concluded that their algal assay was highly efficient in detection of biological potential. It was especially efficient in estimating growth as it related to orthophosphate content of the effluent and receiving water.

Although the algal assay procedure: Bottle Test was developed to detect the nutrient status of natural waters, it has also been used to detect growth-inhibiting substances in natural waters and effluents. Shiroyama et al. (1976) detected inhibition of growth of A. flos-aquae by heavy metals in effluent from a primary sewage treatment plant, and Joubert (1980) demonstrated inhibition of algal growth by water from a leachate site. There is, however, a drawback inherent in this freshwater method. Some liquid waste effluents contain algae that have adapted to living in waste impoundments; therefore when a sample taken at the outfall, may contain

algae that can grow in test medium. Miller et al. (1978) suggested filtration of all wastewater through a 0.45 µm filter to eliminate contaminating algae. Most complex wastes contain suspended particles, however, and pigments of colored wastes are often particulate. For this reason, filtered wastes are often grossly different from the originals, having lost all of the suspended matter, and highly colored wastes may become colorless. We suggest that waste samples should be examined for the presence of algae before a test. If no algae are found, then filtration should not be done. Bacteria in unfiltered waste may flourish under algal culture conditions, and short-term tests of 48 to 72 hrs (described below), may best avoid bacterial effects on algae and toxicants.

Lack of filtration precludes use of the gravimetric method and particle counters for estimation of biomass. However, the bottle test was developed for identification of the nutrient status of natural waters. The end points for such a test must necessarily be different from those of a test to determine bioactivity of complex wastes that contain both growth inhibitors and growth stimulators. Whereas nutrients such as orthophosphate do not break down or attach permanently to flask walls and are thus available for algal growth at all times, toxic organic and inorganic components of complex wastes may be decomposed biologically, adsorb strongly to flask walls, break down in the presence of light required for algal growth, precipate from solution, or form complexes with other substances. Algae may also develop resistance to the toxicants or the ratio of algal biomass to toxicant concentration may increase as the population grows slowly, ultimately resulting in a ratio favorable to rapid algal growth. All of these factors that affect response of algae to complex wastes may operate in long-term

tests, and, therefore, short-term tests are best for estimation of effects on growth. Growth rates or doubling time may be estimated by cell counts with a microscope, optical density measurements, quantitative analysis of chlorophyll, or by fluorescence. Biomass after 14 days of growth is a poor estimate of effects of complex wastes because toxicity is often lost after 5 or 6 days (Walsh et al., in press).

We suggest that an exponential phase plot of algal growth is not needed for toxicity tests. The purpose of such tests is to detect toxicity. Attempts to use them for any other purpose must consider fate of the toxicant, and this is usually impossible in standard tests. Our data show that suppression of growth after 48 or 72 hr exposure is the best indicator of toxicity.

Marine algal assays are similar to those with freshwater species because they are generally in vitro tests on selected, easily-cultured algae. Algal contaminants of liquid waste are seldom a problem in marine tests. Contaminant algae are usually low in number in waste streams, which are generally freshwater. Freshwater algae are killed by the salinity of the marine algal medium, and except for adding very small amounts of nutrients to the medium, have little effect on growth of test species.

Marine algal tests differ from one another mainly in the type of exposure vessel, growth medium, and algal species. Exposure vessels are usually Erlenmeyer flasks (Canterford, 1979; Williamson, 1980) or optically matched test tubes (Ferguson et al., 1976; Walsh and Alexander, 1980). Optically matched test tubes that fit in the cuvette holder of a

spectrophotometer are convenient because they take little space, and population size may be estimated by the absorbance value at any desired wavelength without removing samples from the tubes.

Freshwater algal growth medium is usually prepared by addition of nutrient salts, trace metals, and vitamins to well, distilled, or deionized water. Stein (1973) and Starr (1978) list a large number of liquid media, most of which support growth of many algal species. Few media have been tested for use in toxicity tests, but whatever medium is used, chelators should not be added. In tests with liquid industrial or energy process wastes, test media are prepared by adding nutrients directly to untreated effluent and then diluted with medium prepared with water. Medium prepared with water serves as the control medium. It may be necessary to use a nutritionally dilute medium that supports approximately one-half of the full growth in order to detect growth stimulants.

There are two basic types of marine algal growth medium: completely synthetic medium prepared with distilled or deionized water and salts, trace metals, and vitamins; and natural seawater enriched with nutrient salts, trace metals, and vitamins. Stein (1973) gives methods for preparation of marine algal media. Completely synthetic media may be prepared with commercial salt mix (Walsh and Alexander, 1980) or with reagent grade chemicals and distilled or deionized water (Harrison et al., 1980). Harrison et al. (1980) tested 83 coastal and open-ocean species of algae with their medium and reported that only three did not grow in it.

The most commonly used enriched natural seawater medium is the f/2 formula of Guillard (Guillard and Ryther, 1962; Sears, 1967; Stein, 1973).

Booth (1975) described growth of 26 species of phytoplankton in enriched seawater. Although algae grow well in it, enriched natural seawater, cannot be used in toxicity tests with algae and waste outfalls. Most outfalls are freshwater, and salts must be added to them in order to estimate potential effects when their constituents reach estuarine and oceanic systems.

Test medium for algae is prepared by adding salts, trace metals, and nutrients directly to liquid waste. Salinity may be between 5 and 35 parts per thousand (ppt), depending upon data requirements and species tested. Euryhaline species need to be adapted by at least three serial transfers to each salinity. Waste with added salt is diluted to test concentrations with growth medium prepared with distilled or deionized water. Nutrient strength should be approximately one-half that which supports full growth so that growth stimulation can be detected.

Although most toxicity tests are performed on a single species of alga, it is best to test the most sensitive clones of at least three species in order to evaluate possible effects of toxicants on algae. Algae vary greatly in response to toxicants: e.g., a substance may be toxic to one species and non-toxic to another, whereas, the same two species may respond in an opposite manner to another toxicant (Hollister and Walsh, 1973).

A few tests have been reported on response of mixtures of algal species to toxicants in the laboratory. Dunstan (1975) stated that the most significant force exerted by sewage discharge is to change the pattern of phytoplankton production, distribution, and population dynamics and that ecological interpretation of results based on culture of one or two species can be misleading. Mosser *et al.* (1972) reported that the diatom, *T. pseudonana*, had a more rapid growth rate than the green alga, *D. teriolecta*,

when each was grown singly and in mixed culture. However, in the presence of low concentrations of chlorinated hydrocarbon insecticides, dominance was reversed, with D. tertiolecta having the more rapid growth rate. Green algae are generally much more resistant to toxicants than diatoms (Fisher and Wurster, 1973), and presumably D. tertiolecta had a competitive advantage over T. pseudonana in the presence of chlorinated hydrocarbons. Walsh and Alexander (1980) reported that the diatom, S. costatum, had a more rapid growth rate than the rhodophyte, Porphyridium cruentum, in single-species and mixed cultures. Waste from a paper products plant had no effect on growth of P. cruentum but inhibited growth of S. costatum in single and mixed cultures, resulting in reversal of dominance in mixed culture. On the other hand, Walsh and Alexander (1980) reported that marine species (P. cruentum, Neochloris sp.) sensitive to the herbicide, neburon, were less sensitive when grown in mixed culture with resistant species (Nitzschia angularum, Navicula inserta). In the tests with neburon, it appeared that the less sensitive species protected the more sensitive species by initial rapid growth accompanied by adsorption of the toxicant, which reduced it to less than toxic concentrations. Sanders et al. (1981) exposed natural assemblages of marine phytoplankton to copper, chlorine, and thermal stress and concluded that chronic exposure to low concentrations of pollutants associated with power plant cooling systems can induce substantial shifts in species composition of the assemblages.

Laboratory tests with mixtures of species suggest that toxicants may affect algal population dynamics, resulting in dominance of resistant species. Harrison et al. (1977) showed dominance of copper-resistant species over copper-sensitive species when exposed to copper, and Jensen

et al. (1974) showed that a clone of S. costatum from a zinc-polluted fjord was approximately two orders of magnitude more resistant to zinc than a clone from an unpolluted fjord. The mechanism of resistance, whether simply physiological or genetic, is unknown, but Fisher (1977) suggested that adaptation could involve changes in membrane structure and permeability, thus limiting uptake of the toxicants. Whatever the mechanism of adaptation, pollutants may cause changes in algal community structure with subsequent effects on herbivore populations. Also, resistant species may adsorb toxicants (Walsh, 1977) which, when passed upward in food chains, may accumulate to toxic concentrations. Ohkawa et al. (1980) used a simulated aquarium ecosystem that contained carp, (Cyprinus carpio), daphnids (Daphnia pulex), snails (Cipangopaludina japonica), and a mixture of pond algae to test partitioning of the pesticides DDT and S-fenvalerate. After exposure for seven days, algae were efficient accumulators of the toxicants and the higher organisms obtained them either directly from the water or through food chains (algae $\rightarrow$snails or algae $\rightarrow$daphnids $\rightarrow$fish). These concepts, however consistent with ecological theory, need to be investigated in the field because relationships between laboratory data and field theory have not been described.

Continuous Culture

Algal populations may be grown in continuous culture in which fresh growth medium is introduced at a measured rate and old medium washed from the system at the same rate. Since algal cells are removed from the system in the old medium, rate of flow can be regulated according to rate of growth of the algal population and any number of cells between zero and maximum

standing crop may be maintained. Pavlou (1972) described methods for continuous culture of algae in chemostats, and numerous papers have reported methods for maintaining and testing of algae in chemostats.

Continuous culture techniques may be used to identify sublethal effects of toxicants in long-term tests and offer the opportunity to study algal responses to environmental change under conditions that resemble the natural environment more than batch culture. For example, continuous input of low concentrations of nutrients to a culture in the steady state resembles natural conditions more closely than batch culture, where exponentially growing cells draw upon a large reservoir of nutrients. Also, metabolites that may affect tests are removed from flow-through systems. Sharp et al. (1980) demonstrated that cellular chemistry and population physiology in batch culture are not synchronized, whereas they are in steady-state systems.

A very important advantage of continuous culture over batch culture is related to the fact that many toxicants adsorb to the glass walls of exposure vessels. This could remove toxicants from solution and result in underestimation of toxicity. Continuous culture also allows study of physiological responses in relation to pollution, such as periodicity of metabolic processes (Eppley et al., 1971), nutrient relationships (Davis et al., 1973; Thomas et al., 1980d), effects of temperature on growth, nutrient uptake and chemical composition of algae (Goldman, 1979), carbon budgets (Sharp et al., 1980), and numerous others.

Continuous culture in chemostats may also be used to study effects of pollutants on algal communities. The method is limited, however, to use of species that are compatible with regard to growth rate. For example, the lowest acceptable flow rate would be determined by the doubling time of the slowest-growing species. This might confer considerable competitive advantage to fast-growing species and make toxicity tests useless. Research on development of flow-through systems with algal communities is needed.

Results of batch and continuous flow tests may be confounded if experimental conditions are not controlled carefully. Algal population growth and morphology are determined by many factors, such as temperature (Reynolds et al., 1975; Nakamura and Miyachi, 1980; Terlizzi and Karlander, 1980), nutrients (Paasche, 1971; Turner, 1979; Terlizzi and Karlander, 1980), salinity (Terlizzi and Karlander, 1980), concentration of carbon dioxide (Nakamura and Miyachi, 1980), photoperiod (Chisholm and Costello, 1980), and average cell size (Banse, 1976). Temperature has profound effects on toxicity (Fisher and Wurster, 1973; Cairns et al., 1975: Styron et al., 1976). Although algae require heavy metals in their nutrition, they must be chelated or else they are toxic, causing slowed growth and abnormal morphology. Solvent carriers of toxicants, such as acetone or triethylene glycol, must be tested for toxicity before use with algae. For example, Stratton et al. (1980) demonstrated antagonistic, synergistic, and additive behavior of acetone and the pesticide permethrin in three species of Anabaena. They recommended that solvent sensitivity of each organism be determined because of possible variation in species response and that every combination

of solvent, algal species, and test compound be analyzed to be sure that toxicities obtained are due to the compound and not to its interactions with solvent. In tests on complex industrial and energy process wastes, correct choice of solent is very important in determining toxicities of chemical fractions as discussed later.

It is important, therefore, for maximal protection of aquatic systems, that sensitive algal species be tested under physical and chemical conditions optimal for detection of toxicity or stimulation.

END-POINTS OF ALGAL TOXICITY TESTS

There is a variety of ways by which algal response to pollutants can be measured. Growth response occurs either by enhanced growth or decreased growth relative to solvent control cultures. Complex wastes often contain both growth stimulants and growth inhibitors. Cells can be enumerated with counting chambers and microscopes, but not with electronic particle counters because wastes usually contain particulate matter that blocks the orifice. Particles in effluents also preclude accurate measurement of biomass by gravimetric methods. Total chlorophyll measurements give only an approximate estimate of the amount and physiological state of algal biomass, and by themselves do not give an accurate account of algal response to toxicants. Amount of chlorophyll per cell is a better estimate of effect than total chlorophyll alone. Traditional extractive methods for spectrophotometric quantitation of chlorophyll and their errors were discussed by Harjula (1979), but it should be recognized that occasionally a substance from the

effluent may interfere with the tests. Jeffrey and Humphrey (1975) gave equations for calculating pigment concentrations in various types of algae. Generally, phaeopigment is not detected in appreciable amounts in healthy algal cultures. However, it should be quantified in all algal toxicity tests that use chlorophyll concentration as an end-point. It may be that phaeopigment concentration is as important as that of chlorophyll. Holm-Hansen (1978) described a fluorimetric technique for quantitation of extracted chlorophyll that also has the advantage of simple estimation of phaeopigments. Spectrophotometric determination of phaeopigment requires a neutralization procedure after acidification of chlorophyll, whereas the fluorimetric method does not, with a considerable saving of time.

Fluorescence of intact algal cells has been used extensively to estimate algal biomass. Application of this method was reviewed by Harris (1978). Although results with whole cells from natural systems are more variable than with extracts, in vivo techniques are used extensively in basic and applied research on algae. In the laboratory, in vivo techniques have the great advantage of ease of measurement because time-consuming extraction is not required and environmental factors that cause variability in field populations do not operate under culture condition. However, fluoremetric measurement of chlorophyll in vivo in experimental studies on laboratory populations of algae may lead to erroneous conclusions. Fluorescence yield is a function of chlorophyll concentration and chloroplast shape. Complex wastes from industrial effluents often cause conformational changes in chloroplast structure, and Clement-Metral and Lefort-Tran (1974) showed that changes in thylacoid shape changed the fluorescence emission characteristics of the red alga, P. cruentum.

The rate of photosynthesis per unit biomass, per unit chlorophyll, or by a population has been used to estimate effects of pollutants on algae (Stadnyk et al., 1971; Erickson and Hawkins, 1980). It is unclear whether the ^{14}C method measures net or gross primary production because of possible losses of organic matter by excretion. Estimates of loss of organic matter range from undetectable (Williams and Yentsch, 1976) to more than 75% of photoassimilated carbon (Sharp, 1977), and magnitude of excretion may vary with species and culture conditions. It is generally held that values from the ^{14}C method lie between the true net and gross photosynthesis (Strickland, 1960). The ^{14}C method may be used in toxicity tests if it is known that the toxicants do not affect excretion of photoassimilated carbon. DeFilippis and Pallaghy (1976) showed that zinc and mercury inhibited excretion of glycolate from Chlorella sp., which suggests that treated cultures could not be compared to control cultures in toxicity tests because their rates of excretion were different.

Two methods that may be used for measurement of effects of wastes on algae are the adenylate energy charge and ATP assays. The adenylate energy charge is defined in molar concentrations of the adenine nucleotides as

$$\frac{(\{ATP\} + \frac{1}{2}\{ADP\})}{(\{ATP\} + \{ADP\} + \{AMP\})}$$

Since cellular growth and division utilize energy available in the adenylate pool, Falkowski (1977) suggested that the adenylate energy charge could be used as an indicator of physiological state of S. costatum and demonstrated differences in energy charge between nitrogen-limited continuous and batch cultures in relation to temperature.

Adenosine triphosphate (ATP) degrades rapidly after an organism dies and can be used, therefore, as an indicator of the amount of living biomass in a system (Holm-Hansen and Booth, 1966; Holm-Hansen and Pearl, 1972). ATP analyses have been used to estimate amount of living material in activated sludge (Patterson et al., 1970; Chui et al., 1973) and waste water (Levin et al., 1975). Kennicutt (1980) demonstrated that ATP analysis can be used in routine toxicity tests and may be valuable in assaying impacts of pollutants on aquatic environments.

The adenylate energy charge may be useful in detecting sublethal effects of complex wastes on algae grown in them, and ATP analysis may be useful in defining effects of pollutants over selected time periods on algal populations. However, although both methods are potentially useful in algal toxicity tests, more research is needed to verify their utility and to adapt them to exposure techniques with complex wastes.

In static algal toxicity tests, toxicants are usually added to cultures with low numbers of algae, and population growth is measured over a period of time, often 96 hr. Walsh (unpublished) grew S. costatum for 48 hr in toxicant-free medium and then counted the number of living cells, dead cells, and those of abnormal morphology. Dilutions of the insecticide Dursban were made in triplicte cultures which were then incubated for 48 hr and again enumerated. Table 1 shows that the insecticide inhibited population growth and killed cells. It also caused deformations of cellular morphology at concentrations up to 2.5 parts per million (ppm). Percentage of abnormal cells was greatest at 1.0 ppm. Low numbers of abnormal cells above that concentration were due to rapid death of cells after treatment, as indicated

by cell numbers at 48 and 96 hr.

Studies of effects of complex waste effluents on survival and morphology of algae are important because they suggest mechanisms of action of the toxic factors. Death of cells is easily measured by use of mortal stains (Crippen and Perrier, 1974; Reynolds et al., 1978), and the data for Dursban described above were obtained by use of the mortal stain Evans blue. Presumably, toxicants that kill algae (algicides) are more dangerous than those that only inhibit growth (algistats), but this may not be so if there is constant input of effluent into receiving water.

Fitzgerald and Faust (1963) recommended that toxicants, such as copper compounds, used to control algae should be studied for their algicidal and algistatic properties in order to evaluate optimal conditions for their use in swimming pools, ponds, and lakes. They suggested that algae whose growth was inhibited by toxicants be placed in fresh growth medium. If growth occurred, the toxicant was algistatic; if growth did not occur, it was algicidal. Payne and Hall (1979) gave a refined method for determining if chemicals are algistatic or algicidal. The method works well, but is probably of little use with waste effluents because, as noted above, constant input of toxicants would cause continued reduced numbers unless the algae developed resistance.

A common response of algae to heavy metal and industrial waste pollution is change in cell size and morphology (Nuzzi, 1972; Azam et al., 1973; Davies, 1974; Sunda and Guillard, 1976; Harrison et al., 1977; Thomas et al., 1980a). Falchuck et al. (1975a,b) described the role of zinc in cell size and morphology of Euglena gracilis. Zinc-deficient cells contained double the amount of DNA than did normal cells, and the authors concluded that zinc

deficiency affects early mitotic events. Most studies report large and aberrant cells in the presence of above-normal concentrations of heavy metals. Davies (1976) suggested that large and aberrant cells are caused by the uncoupling of cell growth and cell division. Figure 1 shows aberrant cells of S. costatum produced by growth medium in which the metals were unchelated and by several industrial waste effluents. Abnormalities occurred in cell shape and internal structure. Elongated shape and the presence of indentations on the walls suggest that mitosis occurred but that cytokinesis was inhibited. When abnormal cells were placed in uncontaminated growth medium, only normal cells were seen after two days of growth (Walsh, unpublished). Figure 1 also shows abnormal chloroplasts in exposed cells. Silverberg (1976) demonstrated ultrastructural changes in mitochondria of Ankistrodesmus falcatus, Chlorella pyrenoidosa, and Scenedesmus quadricauda grown in medium with cadmium chloride and concluded that the mitochondrion is the primary target for cadmium-associated toxicity.

Since heavy metals are often major constituents of complex industrial and energy process wastes, they may cause cellular abnormalities that affect the ability of cells to adjust to polluted conditions. Ibrahim (1970) found numerous aberrant cells living near outfalls of textile plants in Egypt. In tests with the mortal stain Evans blue, Walsh (unpublished) found that only apparently normal cells died in the presence of Dursban.

It is possible that the gross morphological changes caused by complex wastes in the laboratory and field are related to physiological and subcellular morphological adjustments to chemical stress. The small amount of data on the subject of adaptation of unicellular algae to chemical stress suggests that genetic selection need not be involved.

Choice of the end-points listed above depends upon requirements of each testing situation, and the strong and weak points of each test must be recognized so as not to draw unwarrented conclusions from the data.

ALGAL BIOASSAYS WITH WHOLE WASTE

Vocke et al. (1980) tested scrubber ash slurry from the settling pond of a coal-fired generating plant against A. falcatus, S. obliquus, S. capricornutum and Microcoleus vaginatus. The slurry was filtered through a 0.45µm porosity filter, mixed with growth medium (EPA, 1971), and filter-sterilized through a 0.22 µm porosity filter. After two weeks of growth, population density was estimated by measurement of chlorophyll a. Calculated concentrations that would inhibit growth by 50% (EC50) were: A. falcatus, 11.3%; S. obliquus, 8.8%; S. capricornutum, 15.4%; and M. vaginatus, 3.0%. The authors concluded that a standard species, such as S. capricornutum, the least responsive species, should be used only for the purpose of quality control and recommended use of several species in each test.

Walsh et al. (1980) tested effects of 23 textile mill wastes on growth of S. capricornutum and S. costatum. The freshwater species was tested with a modified algal assay bottle test (EPA, 1977) that recommended filtration and autoclaving or filtration only of the liquid waste. Growth medium for S. costatum was prepared by adding artificial sea salts and nutrients to raw

waste. This medium was not filtered or sterilized so as not to change any of the dissolved or suspended substances. The method was described by Walsh and Alexander (1980).

All of the textile mill wastes affected growth of both species in one of three ways: stimulation, inhibition, or stimulation at low concentrations and inhibition at higher concentrations (Fig. 2). Autoclaving changed the bioactive properties of some wastes: stimulatory effects of two were reduced, inhibitory effects of three were reduced, and one was changed from highly inhibitory to highly stimulatory.

Walsh and Alexander (1980) showed that when two species of marine algae were grown together in the presence of textile mill waste, their relative numbers were changed drastically. Skeletonema costatum was sensitive to the waste at concentrations between 10 and 20%. Porphyridium cruentum did not respond to 20% waste. When grown together in untreated medium, cell number of S. costatum was approximately twice that of P. cruentum. In 20% waste, however, cell number of P. cruentum was approximately twice that of S. costatum, showing that the waste could potentially cause changes in relative sizes of the populations in algal communities in receiving waters.

Liquid waste effluents from industrial and energy process outfalls are complex mixtures of organic and inorganic substances, including heavy metals. Walsh et al. (1980), in a study of 23 effluents, detected no obvious relationship between bioactivity and color, biological oxygen demand, chemical oxygen demand, or concentrations of sulphide, phenol, suspended solids, cyanide, organic carbon, total organics extracted with methylene chloride, metals, ammonia, nitrate, nitrite, Kjeldahl nitrogen, orthophosphate, or total phosphorus. They stated that bioactivity of a

complex waste is probably related to interactions among the components, with no substance having a dominant effect. Growth stimulation was not correlated with high concentrations of nitrate or phosphate, and growth inhibition was not correlated with concentrations of metals or organic substances. Some heavy metals were in very high concentrations in non-toxic wastes. It is probable that metals were chelated by the organic molecules or that antagonistic behavior of some metals reduced their toxicity.

In the above tests (Walsh et al., 1980), the procedure with S. capricornutum required incubation of the alga with wastes for 14 days, whereas the marine test required only four days. Only 35% of the wastes were toxic to S. capricornutum and 71% were toxic to S. costatum. Walsh et al. (in press) tested S. capricornutum against 10 unfiltered, unautoclaved industrial wastes and found that toxicity was lost in none by the ninth day of exposure, but was lost in nine of the 10 after that time. Since toxicity and stimulation were apparent at four days, the authors recommended that tests be carried out for no more than four days. Eloranta and Laitinen (1981) also recommended a four-day incubation period for detection of algal growth stimulation by effluents from sulphite cellulose and paper factories.

Litton (1979a,b) reported the growth response of S. capricornutum to limestone scrubber samples and symthetic fules. Figure 3B shows the response to extracts of scrubber filter cake. It is shown that there was an initial decrease in the number of cells on the first day in all but one treated culture. Growth of the control population was greater than that of all treated cultures on the fourth day of exposure, but by the 11th day, there was little difference between treated and control cultures.

Figure 3A shows effects of synethetic fuel on growth of S. capricornutum. In this example, growth was inhibited by three concentrations and arrested by one. The three inhibited cultures recovered by the 16th day, when two of them were equal to the control in cell numbers. Growth rate of the third was rapid between days 10 and 16 and its curve was still rising on the 16th day.

These two examples are typical of the toxic response of S. capricornutum to liquid wastes: initial inhibition followed by recovery. Litton (1979a) concluded that the limestone scrubber filter cake extract was not toxic to algae in spite of the fact that growth was suppressed strongly by all concontractions in the early part of the test. The same is true for the synthetic fuel, where growth of algae exposed to 5.6% fuel was strongly suppressed. Litton concluded that growth was impaired only at 18% waste because only data from the 16-day exposure were used.

We believe these data show clearly the inaccuracies inherent in long-term tests with algae. The presence of growth inhibitors was demonstrated within four days in both tests. Algal assays are tests for growth inhibitors and growth stimulators, and they cannot address the problem of fate of toxicants in flasks. It was noted above that toxicants may degrade, adsorb to exposure vessel walls, attain non-toxic biomass to toxicant concentration ratios, etc. Algal toxicity tests cannot address these questions, which can be answered only by studies designed specifically to study fate. Long-term tests often do not identify toxic wastes and are so complicated by considerations of toxicant fate that they are nearly worthless.

Data from algal toxicity tests should be expressed in such a way as to allow comparison of responses to waste streams. For expression of growth stimulation, Walsh et al. (1980) recommended the term SC20 for the calculated concentration that would stimulate growth by 20%. In their tests, the mean upper 95% confidence interval for algal growth in control cultures was 17% above the control mean. Therefore, growth stimulation greater than 17% above control was a significant response, and 20% above control was a convenient value to calculate. The SC20 may be calculated by straight-line graphical interpolation (APHA, 1975) or preferably by more sophisticated methods, such as moving average (Stephan, 1977), that allow calculation of 95% confidence intervals.

The EC50, which is the calculated concentration that would inhibit population growth by 50%, is a standard expression that has been used for many years. It may be calculated by the same methods used for the SC20, and 95% confidence intervals should be reported.

Payne and Hall (1979) recommended that the algistatic concentration that inhibits growth by 95% (AC95) should be used to express effect. This value is based upon the assumption, that, if an algal population in the field is reduced by a waste to 5% of its density before exposure, it will recover to its original density after some unspecified period of time. We are not familiar with field data that support this assumption. Recovery of populations in the field is dependent upon many things, e.g., grazing rates of herbivores, effects of wastes on herbivores and carnivores that eat them, nutrient content of the wastes and receiving water, wastes from other outfalls in the area, competitive ability of all algae in the immediate

vicinity, temperature, etc. Unless recruitment occurs, populations reduced to small numbers tend to disappear.

It cannot be assumed that algae in the field are subjected to a single, evanescent exposure to complex wastes as occurs in laboratory algistatic tests. It is most likely that they are exposed to wastes over protracted periods of time under varying environmental conditions. Given exposure over relatively long periods of time under field conditions, it is likely that algal populations and communities adapt either physiologically or genetically to toxicants. Neither the EC50 nor the AC95 address this aspect of algal population growth dynamics, and it may be an important omission of both approaches.

The AC95 concept is invalid in most tests because of statistical considerations. It is impossible to attain an accuracy of 5% in algal tests because of variability of population growth rates in cultures treated with complex wastes: 95% growth inhibition cannot be distinguished statistically from 90% or 100% inhibition. The method of Payne and Hall (1979) was described with single organic pollutants. Application of the AC95 to industrial waste toxicity is a misuse of the method.

It is sometimes argued (APHA, 1975) that EC50 values should not be used as an expression of effects of toxicants on algal growth because such data are quantitative, i.e., they inhibit population growth, and the number of organisms at the end of the test is greater than at the beginning. This is in contrast to quantal data obtained from tests in which organisms are killed, resulting in fewer organisms at the end of the test than at the beginning. It is argued that statistical evaluations, such as Duncan's

Multiple Range Test or Dunnett's Test, should be used to identify significant differences between growth rates in concentrations of toxicants within a test. However, Hewlett and Plackett (1979) pointed out that quantal and quantitative changes are very closely related because "An individual organism responds quantally if an underlying quantitative change, that is, a gradual response, reaches a certain level of intensity characteristic of that individual organism." They then presented a hypothesis that unified the mathmatical treatments of the two types of responses.

ALGAL BIOASSAYS WITH WASTE FRACTIONS

Algal bioassays with whole waste samples give an estimation of the potential hazard of such wastes to aquatic systems, but they do not aid in identification of the toxic or stimulatory substances. Walsh and Garnas (1979) described a method whereby complex liquid wastes may be fractionated chemically in order to identify the classes of toxicants and stimulants in them (Fig. 4). Fractionation and subfractionation were linked closely to bioassays in order to identify possible bioactive substances.

Table 2 shows growth responses of S. costatum to wastes and their fractions from an oil refinery and a sewage treatment plant. Toxicity and stimulatory ability of the petroleum plant resided in the organic fraction. The neutral subfraction was toxic and the acid subfraction was stimulatory. Analysis of sewage treatment plant waste and its fractions showed that growth stimulants may be present but remain undetected in growth tests with whole waste. The EC50 of the whole waste was 15.4%, but no stimulation of growth

was detected. The organic fraction has no effect on growth. However, the inorganic fraction had an EC50 of 15.5% and an SC20 of 0.4%. When subfractionated, toxicity was in the cation (heavy metals) subfraction and growth stimulation in the anion subfraction.

ALGAL TESTS COMPARED TO OTHER TOXICITY TESTS

Algal data are seldom used for setting water quality standards because algae usually are less sensitive than invertebrates or fishes to individual organic pollutants. Recently, however, it has become clear that algae are usually much more sensitive to complex liquid effluents than animals and other organisms. Table 3 shows results of tests on leachate prepared by two methods from regenerator cyclone and second cyclone wastes of a fluidized bed combustion plant in health effects tests (Ames bacterial mutagenicity and cytotoxicity tests; chinese hamster ovary toxicity test) and ecological tests (Daphnia magna and S. capricornutum) (Eischen and Duke, 1980). It is shown that the leachates were more toxic to S. capricornutum than D. magna in three of the four samples. The Ames mutagenicity and Chinese hamster ovary tests did not detect toxicity, and the Ames cytotoxicity test detected toxicity in two of the four samples.

Litton (1979b) found S. capricornutum, D. magna, and Pimephales promelus (fathead minnow) to be more sensitive than bacteria to nine samples from a synthetic fuels production process in the Ames mutagenicity, Chinese hamster ovary, and rodent (rat) toxicity tests (Table 4). All wastes were highly toxic in all of the algal tests, but not in all of the others.

In other tests, Walsh and Alexander (1980) and Walsh et al. (in press) compared toxicity of numerous liquid wastes to freshwater and marine algae and to freshwater and marine crustaceans and fishes. Acute (96 hr) tests were done on 14 textile effluents and S. capricornutum, Daphnia pulex, and P. promelus of freshwater and S. costatum, Palaemonetes pugio (grass shrimp), and Cyprinodon variegatus (sheepshead minnow) of saltwater. Fishes were least sensitive to the wastes. Skeletonema costatum was the most sensitive species to 12 of the wastes and D. pulex was most sensitive to two of them. However, 96-hr EC50's and SC20's of the algae were always lower than the EC50's of D. pulex. Walsh et al. (in press) reported that wastes from a variety of industrial plant types were always more bioactive toward S. capricornutum or S. costatum than toward D. magna and Mysidopsis bahia (bay mysid). Of 10 wastes tested, all affected the algae, but only three affected survival of M. bahia and two affected survival of D. magna. As examples of relative effect, a waste from a synthetic organic fibers plant had the effects shown in Table 5. The data are typical of comparative studies, and when a waste is stimulatory, the SC20 is usually much lower than the LC50 (calculated concentration that would kill 50% of exposed animals).

Walsh and Garnas (1979) reported on subfractions of nine industrial wastes that were toxic to both S. costatum and M. bahia (Table 6; Fig.5). Toxicity due to heavy metals (cation subfraction) and neutral and acid extractable organics is shown. There was a small loss of toxicity in the fractionation step, but there was clear identification of toxic fractions and subfractions. Subfractions of the organic fraction of the paper products plant were recombined and the original toxicity was obtained.

All of the data we have gathered show that algae are more sensitive than animals to complex wastes in laboratory tests. Algal tests should be used for evaluation of pollution potential for such wastes because aquatic community structure is based upon the types of primary procedures present.

PRACTICAL USE OF ALGAL DATA

Algae have been shown to be sensitive to complex industrial and energy process wastes. They are generally better indicators of potential pollution than animals because they respond to both toxicants and growth stimulators. Tests on whole wastes allow estimation of possible effects in relation to volume of discharge (Walsh et al., 1980). The expression:

$$\text{MEU/day} = \left(\frac{100}{\text{SC20 or EC50}}\right) \text{(discharge rate)}$$

where MEU=million effective units per day, may be used to estimate relative potential impact of a whole waste on a receiving stream. For example, a highly toxic or stimulatory waste emitted in large volume is more likely to have an adverse effect on aquatic systems than is a weakly toxic waste emitted in low volume. Walsh et al. (1980) used this expression to rank textile mills according to their potential danger in screening tests (Table 7.).

SC20 and EC50 values may also be used to calculate the dilution required for each waste to be rendered non-toxic or non-stimulatory. Walsh et al. (in press) reported their use in calculation of the instream waste concentration (IWC), which is an estimate of the amount of dilution that an effluent may be

expected to undergo during continuous release into a receiving stream:

$$IWC(\%) = \frac{Qw}{Qr + Qw} \times 100\%$$

where Qw=volume of discharge, and Qr=the 7-day, 10-year low flow volume of the receiving water. The 7-day, 10-year low flow volume (Qr) required for safety can be calculated from the expression:

$$0.01 \times EC50 \text{ or } SC20 = \frac{Qw}{Qr + Qw} \times 100$$

where 0.01=a safety factor currently used by EPA in IWC calculations for issuance of discharge permits.

Chemical fractionation tests with algae identify requirements for waste treatment. When the chemical nature of toxicants and growth stimulators is known, treatment systems that address specific problems can be devised and the considerable costs associated with treatment of whole waste can be reduced.

SUMMARY OF RECOMMENDATIONS

1. The purpose of algal bioassays is to detect toxicity, and this is best done in tests conducted for between 48 and 96 hr after exposure. After that time, interpretation of results is confounded by questions of toxicant fate, and such questions cannot be addressed in routine bioassays.
2. Relatively few algal species have been screened for use in toxicity tests and those that are used are often chosen for convenience of culture

rather than sensitivity to pollutants. There is a need for screening studies to search for new, more sensitive species that respond to both growth inhibitors and growth stimulators.

3. Use of indigenous species in algal tests may not be protective of the environment because pollutants are transferred easily to other areas. Use of sensitive species is much more important than use of indigenous species.
4. Algal cultures should be examined for number of dead cells to determine if the toxicant exerts an algistatic or an algicidal effect. Also, exposed cells should be examined for mophological deformaties.
5. Because of the highly complex nature of industrial and energy-process effluents, effects upon algae cannot be predicted from chemical composition. It is suggested that effect is the result of additive, synergistic, and antagonistic behavior of the chemicals in relation to physical properties of an effluent.
6. Liquid effluents may be fractionated chemically to determine the nature of growth inhibitors and growth stimulators. Effects on growth may be overlooked if fractions are not tested.
7. Algae are more sensitive to complex wastes than animals and should always be used for evaluation of potential hazard.

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Table 1. Effects of Dursban on survival and morphology of *S. costatum*.

Dursban ppm	Before Treatment			After Treatment		
	Average Cells/mm^3	Dead %	Abnormal %	Average Cells/mm^3	Dead %	Abnormal %
0	588	0.52	0	1,806	0.54	0
0.1	604	0.58	0	1,769	0.53	0.9
0.5	633	0.61	0	1,482	0.96	5.9
1.0	600	0.61	0	1,296	3.3	13.0
2.5	607	0.32	0	682	88	0.8
5.0	595	0.44	0	600	100	0

Table 2. Effects of whole wastes, fractions, and subfractions of an oil refinery and a sewage treatment plant on growth of *S. costatum*.

Oil Refinery

Whole waste: EC50 = 45.3%; SC20 = 7.5%

Inorganic fraction: no effect

Organic fraction: EC50 = 43.5%: SC20 = 8.1%

Acid subfraction: SC20 = 8.6%

Neutral subfraction: EC50 = 84.4%

Base subfraction: no effect

Sewage Treatment Plant

Whole waste: EC50 = 15.4% no SC20

Organic fraction: no effect

Inorganic fraction: EC50 = 15.5%; SC20 = 0.4%

Cation subfraction: EC50 = 16.5%

Anion subfraction: SC20 = 0.9%

Table 3. Effects of solid waste leachate from regenerator and second cyclone samples of a fluidized bed combustion plant (Eischen and Duke, 1980).

Sample/Test	Leachate Method 1	Leachate Method 2
Regenerator cyclone		
D. magna, LC50	15.0%	11.0%
S. capricornutum, EC50	4.6%	0.6%
Ames mutagenicity	Negative	Negative
Ames cytotoxicity	Negative	Toxic
Chinese hamster ovary	Not done	Negative
Second cyclone		
D. magna, LC50	5.5%	67.4%
S. capricornutum, EC50	6.3%	0.4%
Ames mutagenicity	Negative	Negative
Ames cytotoxicity	Negative	Toxic
Chinese hamster ovary	Not done	Negative

Table 4. Effects of nine samples from a synthetic fuels production process in the Ames mutagenicity, Chinese hamster ovary, and rodent toxicity tests, on growth of S. capricornutum, and on survival of D. magna and P. promelus. H=high toxicity; M=moderate toxicity; L=low toxicity; NT=toxicity not detected.

Sample No.	Ames	Chinese hamster	Rodent toxicity	P. promelus	D. magna	S. capricornutum
1	NT	NT	NT	H	H	H
2	NT	H	NT	H	H	H
3	L	H	NT	H	H	H
4	M	-	-	H	H	H
5	M	H	NT	H	H	H
6	-	-	-	H	H	H
7	NT	NT	NT	M	H	H
8	-	-	-	M	H	H
9	NT	NT	NT	L	L	H

Table 5. Effects of waste from a synthetic organic fibers plant and a textile mill on growth of S. capricornutum and S. costatum and on survival of D. magna and M. bahia. T=toxicant present but not quantifiable; NT=toxicity not detected.

	S. costatum		S. capricornutum		D. magna	M. bahia
	SC20	EC50	SC20	SC50	LD50	LD50
Synthetic organic fibers	1.0	21.2	2.0	45.6	44.9	NT
Textile mill	0.5	T	0.01	NT	NT	77.5

Table 6. Subfractions of industrial wastes toxic to S. costatum and M. bahia (Walsh and Garnas, 1979)

Industry	Toxic Subfraction
Titanium oxide	Heavy Metals
Oil refinery	Neutral organic
Tall oil products	Neutral organic
Phosphoric products	Heavy metals
Carpeting	Heavy metals
Amine products	Heavy metals
Textiles finishing plant	Heavy metals
Paper products	Neutral and acid extracted organics
Sewage treatment plant	Heavy metals

Table 7. Ranking of textile mill effluents according to relative impact on receiving water. Test organism = Skeletonema costatum, MEU = million effective units (Walsh et al., 1980).

Waste	Discharge (M^3/Day X 10^3)	Response	% Waste	MEU/Day X 10^{-3}
1	4.5	EC50	9.0	50
2	1.0	SC20	1.5	67
3	3.8	EC50	2.0	190
4	9.5	SC20	1.0	950
5	9.8	SC20	0.5	1,960

Figure 1. Morphological abnormalities of S. costatum caused by unchelated heavy metals and industrial wastes. A - normal cells; B - cells from growth medium without EDTA, showing loss of chlorophyll; C - cells treated with waste from a textile mill, showing cell elongation and indentation of cell wall; D - cells treated with waste from a paint plant, showing round chloroplasts; E - cells treated with waste from a chemical plant, showing elongated cells with band-like chloroplasts; F - cells treated with waste from a textile mill, showing bending of elongated cells.

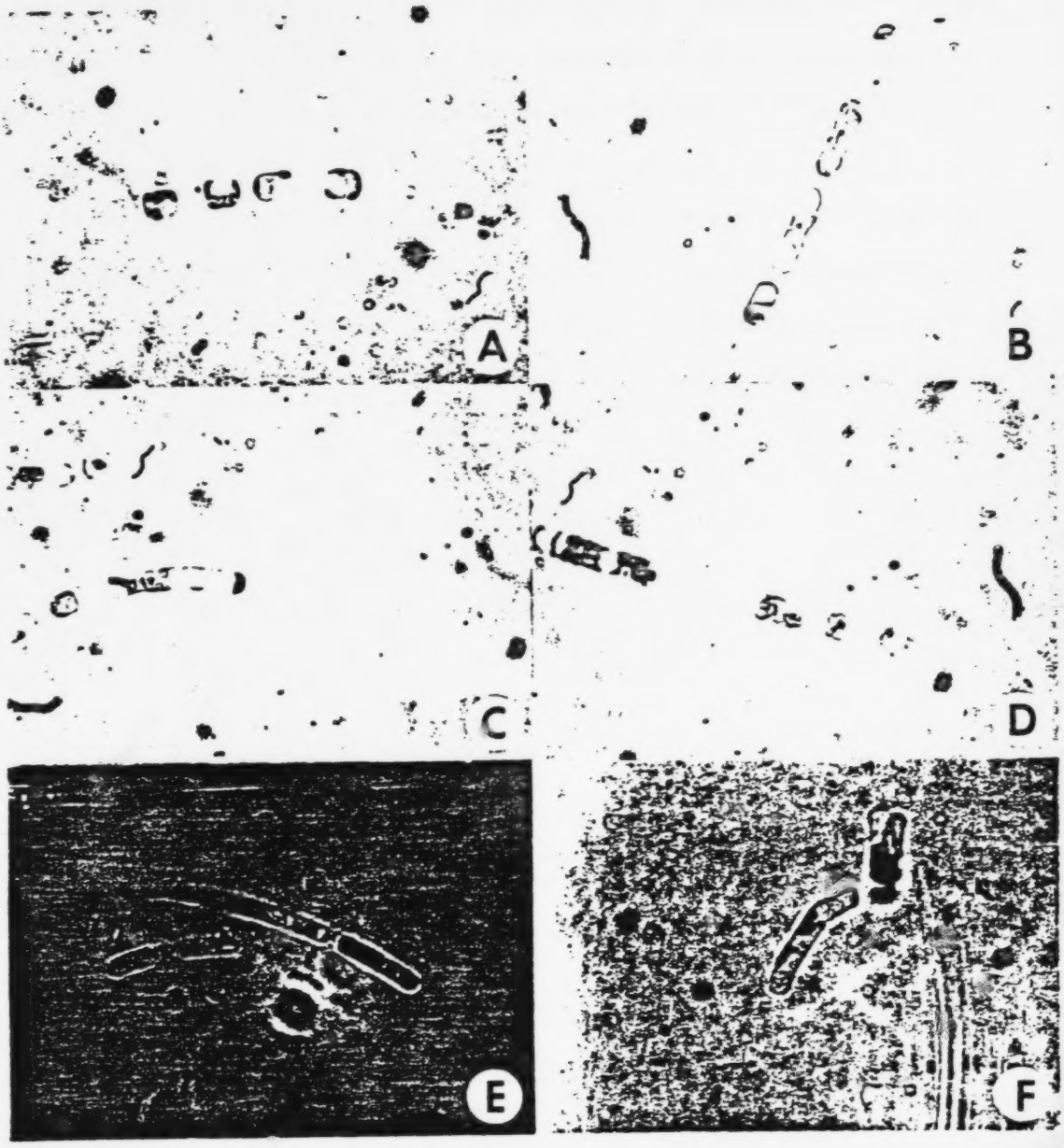

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Figure 2. Effects of textile wastes upon growth of S. costatum. SC20 = calculated concentrated that would stimulate growth by 20%; EC50 = calculated concentration that would inhibit growth by 50% (from Walsh et al., 1980).

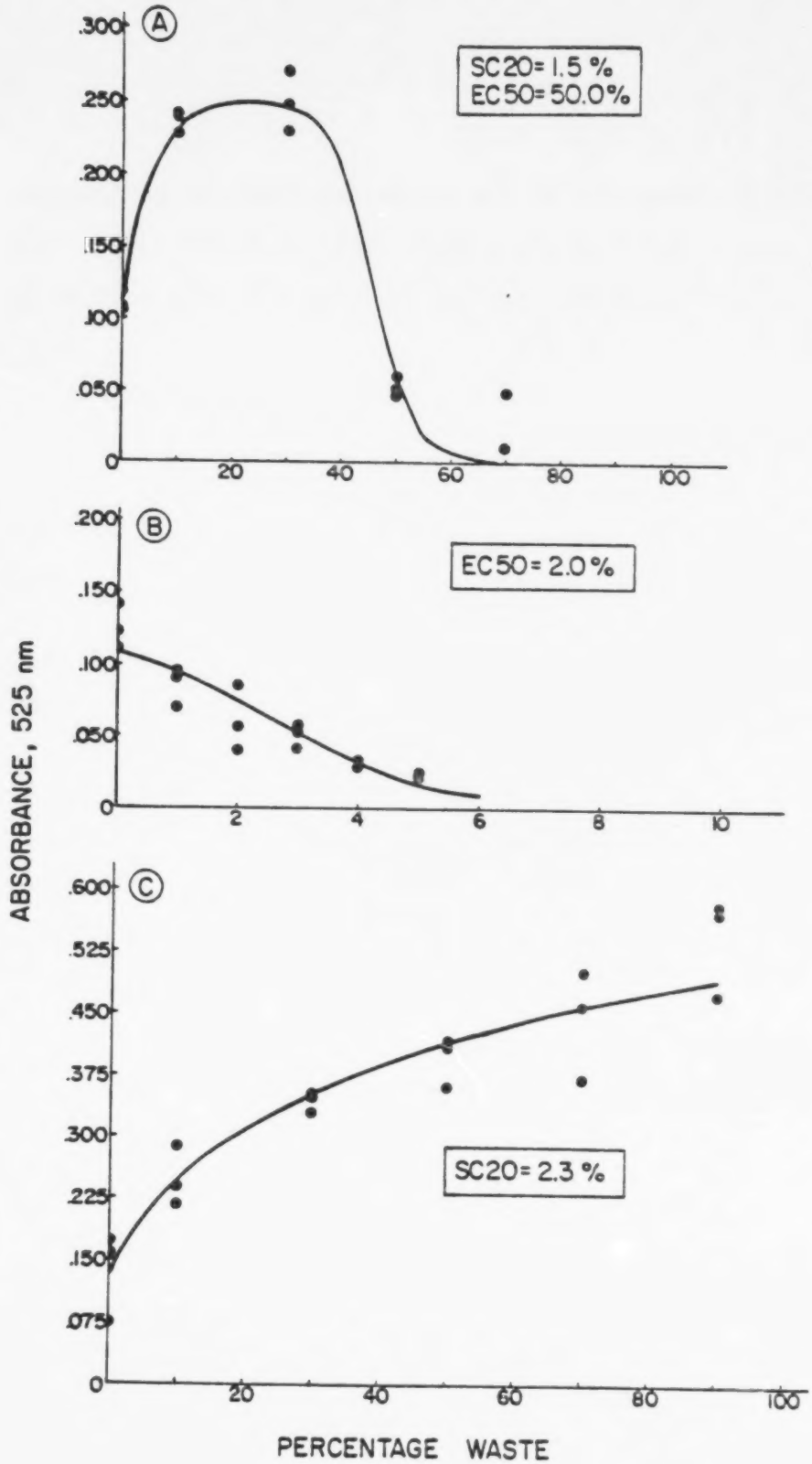

A
SC20= 1.5 %
EC50= 50.0%
B
EC50= 2.0 %
C
SC20= 2.3 %
ABSORBANCE, 525 nm
PERCENTAGE WASTE

Figure 3. Effects of energy process wastes on growth of S. capricornutum. 3A - extract from limestone scrubber cake; 3B - synthetic fuel.

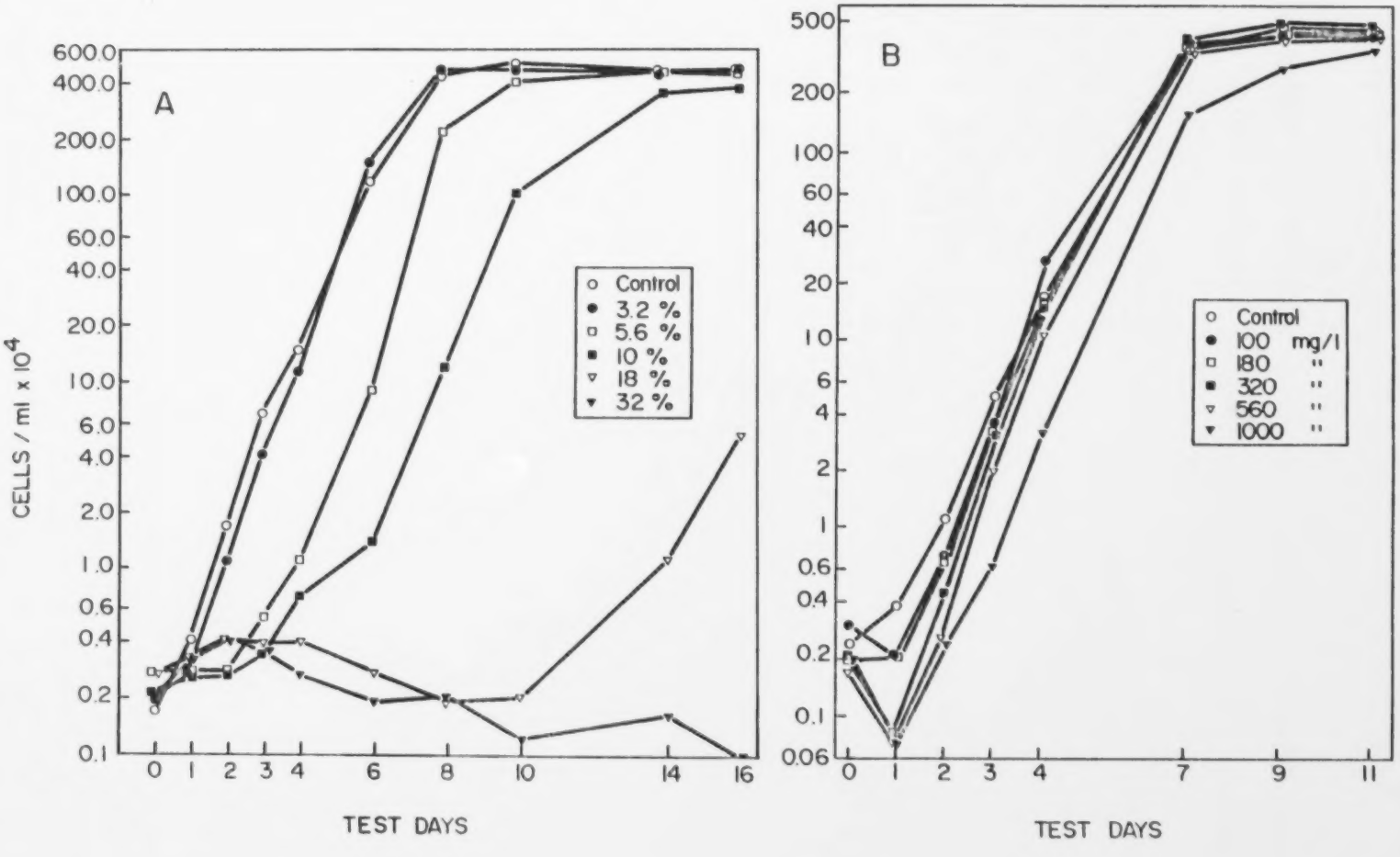

A
B
CELLS / ml x 10⁴
TEST DAYS
TEST DAYS
Control
3.2 %
5.6 %
10 %
18 %
32 %
Control
100 mg/l
180 "
320 "
560 "
1000 "

Figure 4. Integrated chemical fractionation and biological toxicity tests for analysis of complex liquid wastes (Walsh and Garnas (1979).

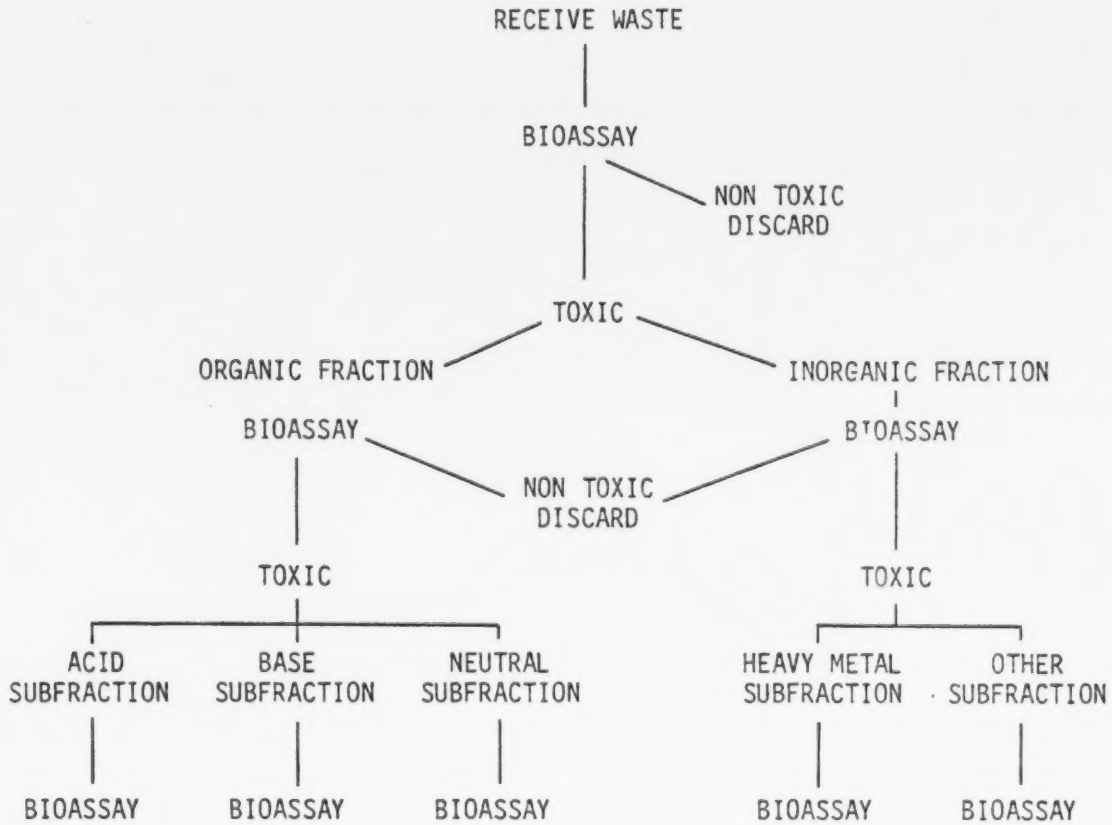
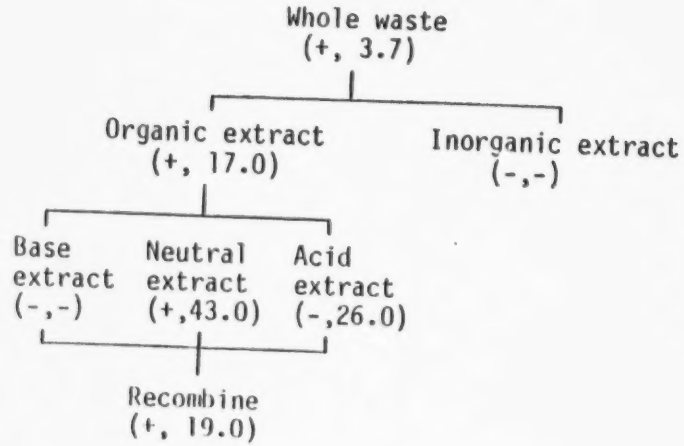

RECEIVE WASTE
BIOASSAY
NON TOXIC
DISCARD
TOXIC
ORGANIC FRACTION
INORGANIC FRACTION
BIOASSAY
BIOASSAY
NON TOXIC
DISCARD
TOXIC
TOXIC
ACID
SUBFRACTION
BASE
SUBFRACTION
NEUTRAL
SUBFRACTION
HEAVY METAL
SUBFRACTION
OTHER
SUBFRACTION
BIOASSAY
BIOASSAY
BIOASSAY
BIOASSAY
BIOASSAY

Figure 5. Effects of industrial wastes and their organic and inorganic fractions and subfractions on growth of S. costatum and survival of M. bahia + = toxic to M. bahia; - = non toxic to M. bahia; numbers are the EC50's (%) for algal toxicity.

PAPER PRODUCTS



SEWAGE TREATMENT PLANT